Guidance for Industry

Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs — General Considerations

DRAFT GUIDANCE

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For questions regarding this draft document contact the CDER Office of Clinical Pharmacology at 301-796-5008 or OCP@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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Biopharmaceutics

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This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance provides recommendations to sponsors and/or applicants planning to include bioavailability (BA) and bioequivalence (BE) information for drug products in investigational new drug applications (INDs), new drug applications (NDAs), and NDA supplements (referred to as the NDA BA and BE Draft Guidance).[2] This guidance contains advice on how to meet the BA and BE requirements set forth in 21 CFR part 320 as they apply to dosage forms intended for oral administration.[3] The guidance may also be applicable to non-orally administered drug products when reliance on systemic exposure measures is suitable to document BA and BE (e.g., transdermal delivery systems and certain rectal and nasal drug products). The guidance should be helpful for applicants conducting BA and BE studies during the IND period for an NDA and also for applicants conducting BE studies during the postapproval period for certain changes to

[1] This guidance was developed by the Office of Clinical Pharmacology, Office of Translational Sciences, and the Office of New Drugs Quality Assessment, Office of Pharmaceutical Science, in the Center for Drug Evaluation and Research (CDER) at the U.S. Food and Drug Administration (FDA).

[2] BA and BE information for drug products in abbreviated new drug applications (ANDAs) and ANDA supplements are not the subject of this guidance. FDA has issued a separate draft guidance on this topic entitled *Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA* (December 2013) (ANDA BE Draft Guidance). The ANDA BE Draft Guidance, when finalized, will represent FDA's current thinking on this topic. Many guidances are referenced throughout this document. The guidance referred to in this footnote, as well as others referenced throughout the remainder of the document, can be found on the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance Web page.

[3] These dosage forms include tablets, capsules, solutions, suspensions, conventional/immediate-release drug products, and modified (extended, delayed)-release drug products.

drug products that are the subject of an NDA.[4] This guidance document is not intended to provide recommendations on studies conducted in support of demonstrating comparability or biosimilarity for biological products licensed under section 351 of the Public Health Service Act.[5]

When finalized, this guidance will revise and replace the parts of FDA's March 2003 guidance for industry on *Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations* (the March 2003 BA and BE Guidance) relating to BA and BE studies for INDs, NDAs, and NDA supplements.[6] Since the March 2003 BA and BE Guidance was issued, FDA has determined that providing information on BA and BE studies in separate guidances according to application type will be beneficial to sponsors and applicants. Thus, FDA is issuing this NDA BA and BE Draft Guidance and, as previously noted, has issued the ANDA BE Draft Guidance for ANDA and ANDA supplements.[7]

We recognize that this guidance cannot address every issue pertaining to the assessment of BA or BE studies for INDs and NDAs, so we suggest sponsors and applicants contact the appropriate review division for guidance on specific questions not addressed by this guidance.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance documents means that something is suggested or recommended, but not required.

II. BACKGROUND

[4] *Bioequivalence* is a statutory term reflected in the Federal Food, Drug, and Cosmetic Act (FD&C Act) in section 505(j) (21 U.S.C. 355(j)), which requires ANDA applicants to demonstrate, among other things, that the proposed generic product is bioequivalent to its reference listed drug. Section 505(j)(2)(A)(iv) of the FD&C Act; see also section 505(j)(8) of the FD&C Act. There is no similar statutory requirement for an NDA applicant either under section 505(b)(1) or (b)(2) of the FD&C Act to demonstrate bioequivalence of its proposed product to another product. As a scientific matter, however, the same or a similar showing of the bioavailability of two products in the NDA context may be needed for the purposes of evaluating the safety or effectiveness of a product. For ease of the reader, we refer to such evaluations of the relative bioavailability for two or more products as an evaluation of bioequivalence in this guidance.

[5] For information on these types of studies, see FDA's Drugs guidance Web page. See footnote #2 for information on accessing this Web page.

[6] Revisions to the March 2003 BA and BE Guidance include (1) expansion of the section on modified-release products, (2) addition of a section on concomitant administration of drug products and combination drug products, (3) addition of a section on alcoholic beverage effects on modified-release dosage forms, (4) addition of an endogenous substance section, (5) addition of a section on drug products with high intrasubject variability, and (6) removal of references to BE studies conducted for ANDAs. The guidance also makes other revisions for clarification.

[7] See footnote #2.

BA assessment of formulations is a component of new drug development. The approaches of evaluating BA and BE discussed in this guidance are designed to aid FDA evaluation of the safety and effectiveness of a product that is the subject of an IND, NDA, or NDA supplement. In this endeavor, we use the totality of information available in the submission, which includes, among other things, information gathered using the principles of BE, exposure-response evaluations, and clinical trial results. The evaluation of BE in the generic drug context, by contrast, is used to support a determination that a generic product may be substituted for its reference listed drug, and involves consideration of different types of data permitted in an ANDA. Accordingly, the approaches discussed in this guidance may differ from similar discussions of BE in the ANDA BE Draft Guidance. For example, this NDA BA and BE Draft Guidance recommends assessment of the effect of food on BA using the approaches set forth in FDA's 2002 guidance for industry on *Food-Effect Bioavailability and Fed Bioequivalence Studies* (the 2002 Food-Effect Guidance). Fasting BE studies generally are sufficient, given the totality of information we consider in evaluating INDs, NDAs, or NDA supplements. In contrast, we recommend in the ANDA BE Draft Guidance fed and fasting BE studies that will provide specific information to support a demonstration of BE under section 505(j) of the FD&C Act, and in turn, to support substitutability. Even though the ANDA BE Draft Guidance revises and replaces the parts of the 2002 Food-Effect Guidance pertaining to ANDAs and ANDA supplements, this NDA BA and BE Draft Guidance does not replace the 2002 Food-Effect Guidance relating to studies for INDs, NDAs, and NDA supplements.[8]

A. General

Studies to measure BA and/or establish BE of a product are important elements in support of INDs, NDAs, and NDA supplements. *Bioavailability* means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action (21 CFR 320.1(a)). BA data provide an estimate of the fraction of the drug absorbed, as well as provide information related to the pharmacokinetics of the drug.

Bioequivalence means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (21 CFR 320.1(e)). Studies to establish BE between two products are important for certain formulation or manufacturing changes occurring during the drug development and postapproval stages. In BE studies, the exposure profile of a test drug product is compared to that of a reference drug product.

B. Bioavailability

BA for a given formulation provides an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation. BA for orally administered drug products can be documented by comparing a systemic exposure profile to that of a suitable reference product. A profile can be generated by measuring the concentration of active

[8] Accordingly, we are in the process of revising the 2002 Food-Effect Guidance.

ingredients and/or active moieties over time and, when appropriate, active metabolites over time in samples collected from the systemic circulation. Systemic exposure profiles reflect both release of the drug substance from the drug product and a series of possible presystemic/systemic actions on the drug substance after its release from the drug product.

FDA's regulations at 21 CFR 320.25 set forth guidelines for in vivo BA studies. As provided in this regulation, the reference product for BA studies should be a solution, suspension, or intravenous (IV) dosage form (21 CFR 320.25(d)(2) and (3)). The purpose of conducting a BA study with an oral solution as a reference is to assess the impact of formulation on BA. Conducting a BA study with an IV reference enables assessment of the impact of route of administration on BA and defines the absolute BA of the drug released from the drug product.

C. Bioequivalence

As noted previously, both BA and BE focus on the release of a drug substance from a drug product and subsequent absorption into systemic circulation. As a result, we recommend that approaches to determining BE generally follow approaches similar to those used for BA. Demonstrating BE involves a more formal comparative test that uses specific references with specified criteria for comparisons and predetermined BE limits for such criteria.

1. Preapproval Changes

BE documentation can be useful during the IND period to compare (1) early and late clinical trial formulations; (2) formulations used in clinical trials and stability studies, if different; (3) clinical trial formulations and to-be-marketed drug products, if different; and (4) product strength equivalence, as appropriate. In each comparison, the new formulation, formulation produced by the new method of manufacture, or new strength is the candidate, or test product and the prior formulation, prior method of manufacture, or prior strength is the reference product. The decision to document BE during drug development is generally left to the judgment of the sponsor, using the principles of relevant guidances (in this guidance, see sections II.C.2, Postapproval Changes, and III.D, In Vitro Studies) to determine when changes in components, composition, and/or method of manufacture suggest that further in vitro and/or in vivo studies be performed.

2. Postapproval Changes

In the presence of certain major changes in components, composition, manufacturing site, and/or method of manufacture after approval, FDA recommends that in vivo BE be demonstrated for the drug product after the change in comparison to the drug product before the change. Under section 506A(c)(2) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 356a(c)(2)), certain postapproval changes that require completion of studies must be submitted in a supplement and approved by FDA before distributing a drug product made with the change.

Information on the types of recommended in vitro dissolution and in vivo BE studies for immediate-release and modified-release drug products approved as NDAs for specified postapproval changes is provided in the following FDA guidances*:*

- *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Control; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*
- *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*

3. BE Considerations

BE studies are usually conducted using a crossover design. For such studies, intrasubject variability should be considered when determining the study sample size. In cases when a parallel design is necessary to evaluate BE, consideration should be given to total variability, including intersubject variability instead of just intrasubject variability.

A test product might fail to demonstrate bioequivalence because it has measures of rate and/or extent of absorption compared to the reference product outside acceptable higher or lower limits. For example, when the test product results in a systemic exposure that is significantly higher than that of the reference product, the concern is the typically limited experience from a safety standpoint for higher systemic concentrations. When the test product has a systemic exposure that is significantly lower than that of the reference product, the concern is potentially a lack of therapeutic efficacy of the test product. When the variability of the test product is greater than the reference product, the concern relates to both safety and efficacy, because it may suggest that the performance of the test product is not comparable to the reference product, and the test product may be too variable to be clinically useful.

When BE is not demonstrated, the sponsor should demonstrate that the differences in rate and extent of absorption do not significantly affect the safety and efficacy based on available dose-response or concentration-response data. In the absence of this evidence, failure to demonstrate BE may suggest that the test product should be reformulated, or the method of manufacture for the test product should be changed, or additional safety or efficacy data may be needed for the test product. In some cases, conclusions of BE based on the peak drug concentration (C_{max}) and area under the plasma concentration time curve (AUC) between the test product and the reference product may be insufficient to demonstrate that there is no difference in safety or efficacy if the systemic concentration-time profiles of the test product and the reference product are different (e.g., time to reach peak drug concentration (T_{max}) is different). For example, differences in the shape of the systemic concentration profile between the test and reference products could imply that the test product may not produce the same clinical response as the reference product. In such cases, additional data analysis (e.g., partial AUCs), exposure-response evaluation, or clinical studies may be recommended to evaluate the BE of the two products.

III. METHODS TO DOCUMENT BA AND BE

Under FDA's regulations, applicants must use the most accurate, sensitive, and reproducible method available to demonstrate BA or BE of a product (21 CFR 320.24(a)). As noted in 21 CFR 320.24, several in vivo and in vitro methods can be used to measure BA and to establish BE. These include, in general order of preference, pharmacokinetic (PK) studies, in vitro tests predictive of human in vivo BA (in vitro-in vivo correlation), pharmacodynamic (PD) studies, studies with clinical benefit endpoints, and other in vitro studies. In addition, where in vivo data are appropriate to demonstrate BA, our regulations provide guidelines on specific types of in vivo BA studies (see 21 CFR 320.25 through 320.29). This guidance predominantly focuses on the use of PK studies to document BA or BE.

A. Pharmacokinetic Studies

1. General Considerations

FDA's regulations generally define BA and BE in terms of rate and extent of absorption of the active ingredient or moiety to the site of action.[9] For in vivo studies, the regulations also provide for use of PK measures in an accessible biological matrix such as blood, plasma, and/or serum to indicate release of the drug substance from the drug product into the systemic circulation.[10] BA and BE frequently rely on PK measures such as AUC to assess extent of systemic exposure and C_{max} and T_{max} to assess rate of systemic absorption. PK-based comparisons to describe relative BA or make BE determinations are predicated on an understanding that measuring the active moiety or ingredient at the site of action is generally not possible and on an assumption that some relationship exists between the efficacy/safety and concentration of the active moiety and/or its important metabolite(s) in the systemic circulation. A typical study is conducted as a crossover study. The crossover design reduces variability caused by patient-specific factors, thereby increasing the ability to discern differences because of formulation.

2. Pilot Study

If the sponsor chooses, a pilot study in a small number of subjects can be carried out before proceeding with a full-scale BA or BE study. The pilot study can be used to validate analytical methodology, assess PK variability, determine sample size to achieve adequate power, optimize sample collection time intervals, and determine the length of the washout period needed between treatments. For example, for conventional immediate-release products, careful timing of initial samples may avoid a subsequent finding in a full-scale study that the first sample collection occurs after the C_{max}. For modified-release products, a pilot study can help determine the sampling schedule needed

[9] 21 CFR 320.1(a) and (e).

[10] See, e.g., 21 CFR 320.24(b)(1)(i). If serial measurements of the drug or its metabolites in plasma, serum, or blood cannot be accomplished, then measurement of urinary excretion can be used.

to assess lag time and dose dumping. The results of a pilot study can be used as the sole basis to document BA or BE provided the study's design and execution are suitable and a sufficient number of subjects have completed the study.

3. Full-Scale Study

General recommendations for a standard BA or BE study based on PK measurements are provided in Appendix A. Nonreplicate crossover study designs are recommended for BA and BE studies of immediate-release and modified-release dosage forms. However, sponsors and/or applicants have the option of using replicate designs for BE studies. Replicate crossover designs are used to allow estimation of (1) within-subject variance for the reference product, or for both the test and reference products, and (2) the subject by formulation interaction variance component. This design accounts for the inter-occasion variability that may confound the interpretation of a BE study as compared to a non-replicate crossover approach. The recommended method of analysis for nonreplicate or replicate studies to evaluate BE is average BE, as discussed in section IV. Recommendations for conducting and evaluating replicate study designs can be found in the FDA guidance for industry *Statistical Approaches to Establishing Bioequivalence.*

4. Study Population

Subjects recruited for BA or BE studies should be 18 years of age or older and capable of giving informed consent. In general, BA and BE studies should be conducted in healthy volunteers if the product can be safely administered to this population. A study in healthy volunteers is likely to produce less PK variability compared with that in patients with potentially confounding factors such as underlying and/or concomitant disease and concomitant medications. Male and female subjects should be enrolled in BA and BE studies unless there is a specific reason to exclude one sex. Such exclusions could be related to the drug product being indicated in only one sex or a greater potential for adverse reactions in one sex compared to the other. For example, oral contraceptives are evaluated in female subjects because the indication is specific to females. If a drug has the potential to be a teratogen, the drug product should be evaluated in male subjects. Female subjects enrolled in the study should not be pregnant at the beginning of the study and should not become pregnant during the study. In some instances (e.g., when safety considerations preclude use of healthy subjects), it may be necessary to evaluate BA and BE in patients for whom the drug product is intended. In this situation, sponsors and/or applicants should attempt to enroll patients whose disease process is expected to be stable for the duration of the study.

5. Single-Dose and Multiple-Dose (Steady State) Testing

This guidance generally recommends single-dose PK studies to assess BA and BE because they are generally more sensitive than steady-state studies in assessing rate and extent of release of the drug substance from the drug product into the systemic circulation.

FDA's regulations at 21 CFR 320.27 provide guidelines on the design of a multiple-dose in vivo BA study. This regulation also identifies instances in which multiple-dose BA studies may be required:

i. There is a difference in the rate of absorption but not in the extent of absorption.
ii. There is excessive variability in bioavailability from subject to subject.
iii. The concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in the blood resulting from a single dose is too low for accurate determination by the analytical method.
iv. The drug product is an extended-release dosage form.[11]

We recommend that if a multiple-dose study design is performed, appropriate dosage administration and sampling be carried out to document attainment of steady state.

6. Bioanalytical Methodology

We recommend that sponsors ensure that bioanalytical methods for BA and BE studies be accurate, precise, specific, sensitive, and reproducible. A separate FDA guidance, *Bioanalytical Method Validation*, is available to assist sponsors in validating bioanalytical methods.[12]

7. Administration Under Fasted/Fed Conditions

The BA or BE study should be conducted under fasting conditions (after an overnight fast of at least 10 hours) except when tolerability issues are anticipated with fasting. In these cases, we recommend that applicants conduct only a fed study. A separate FDA guidance, *Food-Effect Bioavailability and Fed Bioequivalence Studies* is available to assist sponsors.

8. Moieties to Be Measured

The active ingredient that is released from the dosage form or its active moiety and, when appropriate, its active metabolites[13] should be measured in biological fluids collected in BA studies.

Measurement of the active ingredient or the active moiety, rather than metabolites, is generally recommended for BE studies because the concentration-time profile of the active ingredient or the active moiety is more sensitive to changes in formulation performance than that of the metabolite, which is more reflective of metabolite formation, distribution, and elimination. The following are instances when an active metabolite(s) should be measured.

[11] 21 CFR 320.27(a)(3).
[12] See also 21 CFR 320.29.
[13] See 21 CFR 320.24(b)(1)(i).

- Measurement of a metabolite(s) is necessary when the active ingredient or the active moiety concentrations are too low to allow reliable analytical measurement in blood, plasma, or serum. In this case, the metabolite should be measured in lieu of the active ingredient or active moiety. We recommend that the confidence interval approach be applied to the metabolite data obtained from these studies.

- Measurement of a metabolite(s) is necessary in addition to the active ingredient or active moiety if the metabolite is formed by presystemic metabolism and contributes meaningfully to efficacy and/or safety. The confidence interval approach should be used for all moieties measured. However, the BE criteria are only generally applied to the active ingredient or active moiety. Sponsors should contact the appropriate review division to determine which moieties should be measured.

9. Pharmacokinetic Measures of Systemic Exposure

This guidance recommends that systemic exposure measures be used to evaluate BA and BE. Exposure measures are defined relative to peak, partial, and total portions of the plasma, serum, or blood concentration-time profile, as describe here:

- Peak Exposure

We recommend that peak exposure be assessed by measuring the C_{max} obtained directly from the systemic drug concentration data without interpolation. The T_{max} can provide important information about the rate of absorption. The first point of a concentration-time curve based on blood and/or plasma measurements is sometimes the highest concentration, which raises a question about the measurement of true C_{max} because of insufficient early sampling times. A carefully conducted pilot study may help to avoid this problem. Collection of an early time point between 5 and 15 minutes after dosing followed by additional sample collections (e.g., two to five) in the first hour after dosing may be sufficient to assess early peak concentrations. If this sampling approach is followed, we consider the data to be adequate, even when the highest observed concentration occurs at the first time point.

- Total Exposure (Extent of Absorption)

For single-dose studies, we recommend that the measurement of total exposure be:

- Area under the plasma, serum, or blood concentration time curve from time zero to time t (AUC_{0-t}), where t is the last time point with a measurable concentration.

- Area under the plasma, serum, or blood concentration time curve from time zero to time infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$. C_t is the last measurable drug concentration and λ_z is the terminal or elimination rate constant calculated according to an appropriate method.

- For drugs with a long half-life, truncated AUC can be used (see section VII.D, Long-Half-Life Drugs).

For steady-state studies, we recommend that the measurement of total exposure be the area under the plasma, serum, or blood concentration time curve from time zero to time tau over a dosing interval at steady state ($AUC_{0\text{-}tau}$), where tau is the length of the dosing interval.

- Partial Exposure

For orally administered drug products, BA and BE can generally be demonstrated by measurements of peak and total exposure. For certain classes of drugs and under certain circumstances (e.g., to assess onset of an analgesic effect), an evaluation of the partial exposure could be used to support the performance of different formulations by providing further evidence of therapeutic effect. This guidance recommends the use of partial AUC as a partial exposure measure. The time to truncate the partial area should be related to a clinically relevant PD measure. We also recommend that sufficient quantifiable samples be collected to allow adequate estimation of the partial area. For questions on the suitability of the PD measure or use of partial exposure in general, we recommend that sponsors and/or applicants consult the appropriate review division.

10. Comparison of PK measures in BE studies

An equivalence approach is recommended for BE comparisons. The recommended approach relies on (1) a criterion to allow the comparison, (2) a confidence interval for the criterion, and (3) a BE limit. Log-transformation of exposure measures before statistical analysis is recommended. This guidance recommends use of an average BE criterion to compare systemic exposure measures for replicate and nonreplicate BE studies of both immediate- and modified-release products. For additional information on data analysis, refer to Appendix A and to the FDA guidance for industry on *Statistical Approaches to Establishing Bioequivalence.*

B. Other Approaches to Support BA/BE

In certain circumstances, other approaches are recommended to support a demonstration of BA/BE. Below are some general considerations regarding these other approaches. Sponsors should consult FDA's guidances for industry for additional information on these methods as well.[14]

1. In Vitro Tests Predictive of Human In Vivo BA

1.

[14] See footnote 2.

In vitro-in vivo correlation (IVIVC) is an approach to describe the relationship between an in vitro attribute of a dosage form (e.g., the rate or extent of drug release) and a relevant in vivo response (e.g., plasma drug concentration or amount of drug absorbed). This model relationship facilitates the rational development and evaluation of extended-release dosage forms. Once an IVIVC is validated, the in vitro test serves as a surrogate for BA and/or BE testing, as well as a tool for formulation screening and setting of the dissolution/drug-release acceptance criteria.

Specifically, in vitro dissolution/drug-release characterization is encouraged for all extended-release product formulations investigated (including prototype formulations), particularly if in vivo absorption characteristics are being defined for the different product formulations. Such efforts may enable the establishment of an IVIVC. When an IVIVC or association is established (21 CFR 320.24(b)(1)(ii)), the in vitro test can serve not only as a quality control specification for the manufacturing process, but also as an indicator of how the product will perform in vivo.

Additional information on the development and validation of an IVIVC can be found in the FDA guidance for industry *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations*.

2. Pharmacodynamic Studies

PD studies are not recommended for orally administered drug products when the drug is absorbed into systemic circulation and a PK approach can be used to assess systemic exposure and evaluate BA or BE. PK endpoints are preferred because they are generally the most accurate, sensitive, and reproducible approach. However, in instances where a PK endpoint is not possible, a well-justified PD endpoint can be used to demonstrate BA or BE.

3. Comparative Clinical Studies

Clinical endpoints can be used in limited circumstances, for example, for orally administered drug products when the measurement of the active ingredients or active moieties in an accessible biological fluid (PK approach) or PD approach is not possible. Because these circumstances do not occur very often, use of this approach is expected to be rare.

4. In Vitro Studies

Under certain circumstances, BA and BE can be evaluated using in vitro approaches (e.g., dissolution/drug-release testing) during the preapproval and postapproval phases (see 21 CFR 320.24(b)(5) and (6)). For example, orally administered drugs that are highly soluble and highly permeable, and for which

the drug product is rapidly dissolving, documentation of BE using an in vitro approach (dissolution/drug-release studies) may be appropriate based on the Biopharmaceutics Classification System.[15]

The following FDA guidances provide recommendations on the development of dissolution methodology, setting specifications, and the regulatory applications of dissolution testing:

- *Dissolution Testing of Immediate-Release Solid Oral Dosage Forms*
- *Extended-Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations*

In addition, we recommend that sponsors consult other FDA guidances for additional information on when in vitro data may be appropriate to demonstrate BA or BE of a product.

IV. DOCUMENTING BA AND BE FOR VARIOUS DOSAGE FORMS

This section summarizes the recommendations for documenting BA and BE studies based on the specific dosage forms and whether these evaluations occur preaapproval or postapproval.

A. Solutions and Other Solubilized Dosage Forms

For oral solutions, elixirs, syrups, tinctures, or other solubilized forms, in vivo BA and/or BE are generally self-evident and a requirement of in vivo data for a product may be waived (21 CFR 320.22(b)(3)). In such instances, the applicant would be deemed to have complied with and fulfilled any requirement for in vivo data.[16] Although a comparative study is not necessary, characterization of the pharmacokinetics of the drug is required (21 CFR 314.50(d)(3)). In addition, in vivo BE studies that compare different solution formulations are waived based on the assumptions that release of drug substance from the drug product is self-evident and that the solutions do not contain any excipients that significantly affect drug absorption. However, there are certain excipients that may alter the BA (e.g., sorbitol may reduce the BA of drugs, and vitamin E may enhance the BA) in amounts sometimes used in oral liquid dosage forms. In this case, evaluation of in vivo BA and/or BE may be required.

B. Immediate-Release Products

Included in this discussion are capsules, tablets (including conventional, buccal, chewable, orally disintegrating, and sublingual dosage forms), and suspensions.

[15] See the FDA guidance for industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*. This document provides complementary information on the Biopharmaceutics Classification System (BCS).

[16] See 21 CFR 320.22(b)(3).

1. Preapproval Changes

For BA and BE studies, we recommend a single-dose, fasting study be performed. Under certain circumstances, multiple-dose BA studies (see section III.A.5) and/or food effect studies may be necessary (See the FDA guidance for industry *Food-Effect Bioavailability and Fed Bioequivalence).* Unconventional dosage forms (buccal, chewable, orally disintegrating, and sublingual dosage forms) should be administered according to intended label use/instructions. In addition, a BA study may be needed with the unconventional dosage form swallowed intact to assess the impact of accidental swallowing of the intact product. Sampling should adequately capture the T_{max} and C_{max} in addition to total exposure.

We recommend that in vitro dissolution be evaluated for all orally administered products. In vitro dissolution test conditions could be the same or different for unconventional compared to conventional dosage forms. If differences in dissolution data exist, they should be discussed with the appropriate review division.

2. Postapproval Changes

Information on the types of in vitro dissolution and in vivo BE studies needed for approved immediate-release drug products when postapproval changes are made is provided in an FDA guidance for industry entitled *SUPAC-IR: Immediate Release Solid Oral Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. We recommend that for postapproval changes, the in vitro or in vivo comparison be made between the post-change and pre-change products.

C. Modified-Release Products

Modified-release (MR) products include extended-release (controlled-release, sustained-release)[17] and delayed-release products.

Extended-release (ER) products are dosage forms that are designed to extend or prolong the release of active ingredient or active moiety from the drug product and may allow a reduction in dosing frequency as compared to when the drug is administered in an immediate-release (IR) dosage form. These drug products can be developed to reduce fluctuations in plasma concentrations when compared to an IR product. ER products can be capsules, tablets, granules, pellets, or suspensions.

Delayed-release (DR) drug products are dosage forms that release active ingredient or active moiety at a time later than immediately after administration (i.e., these drug products exhibit a lag time in quantifiable plasma concentrations). Typically, coatings (e.g., enteric coatings) are

[17] For the purpose of this guidance, the terms *extended*, *controlled*, and *sustained* are used interchangeably.

used to delay the release of the drug substance until the dosage form has passed through the acidic medium of the stomach. Generally, DR products are treated as IR products. However, if the DR product has complex release characteristics, the relevant review division should be contacted for additional guidance.

If the drug product is an ER product, the following recommendations apply.

1. Preapproval: BA and BE Studies

FDA's regulations at 21 CFR 320.25(f) address the purpose of a BA study for an extended-release product, which is to determine if certain delineated conditions are met.[18] This regulation also provides that "the reference material(s) for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the extended release claims made for the drug product."[19] Appropriate reference products may include (1) a solution or suspension of the active drug ingredient or therapeutic moiety, (2) a currently marketed non-controlled-release drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling of the non-controlled release drug product, and (3) a currently marketed ER drug product subject to an approved full NDA containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling of currently marketed ER product.[20]

In general, the PK profile of the ER product may not match that of the approved IR product (e.g., T_{max} is different) or, in some cases, to another ER product. In such a case, establishing similar PK profiles using C_{max} and AUC may not be sufficient to show that the ER product is bioequivalent to the IR product. Thus, additional safety or efficacy studies or PK/PD assessments may be recommended. This guidance recommends that the following BA studies and food effect BA studies be conducted for an ER drug product submitted as an NDA for the scenarios described below:

<u>New ER formulation comparison to an already-approved IR product</u>

- For drugs with linear pharmacokinetics over the therapeutic dose range: A fasting study should be conducted comparing the ER product administered as a single dose at the highest strength to the IR reference administered over the least common time interval to achieve equivalent total dose as for the ER product.[21] If

[18] 21 CFR 320.25(f)(1).

[19] 21 CFR 320.25(f)(2).

[20] 21 CFR 320.25(f)(2)(i), (ii), and (iv). We recommend that a sponsor seeking to use as a reference product "a currently marketed extended release drug product subject to an approved full new drug application containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling proposed for the extended release drug product," under 21 CFR 320.25(f)(2)(iii), consult with the Agency before commencing such a study.

[21] For example, when a 150-milligram (mg) ER product administered once daily (QD) is being developed that gives an approved 50-mg IR reference product administered three times a day (TID) or a 75-mg product administered two times a day (BID), a comparison of the 150-mg ER product administered as a single dose could be compared to

for safety reasons the highest strength cannot be used, a lower strength may be acceptable.

- For drugs with nonlinear pharmacokinetics over the therapeutic dose range: At a minimum, a single dose of the highest and lowest strengths of the ER product should be compared to their corresponding IR references administered over the ER dosing interval. If the relative BA of intermediate ER strengths cannot be inferred based on the above studies, a single-dose fasting study for the intermediate strength(s) of the ER product should be compared to the corresponding IR reference administered over the ER dosing interval.

- When the ER strengths are not proportionally similar in composition, a single-dose fasting dosage strength equivalence assessment study[22] or a dosage strength proportionality study[23] for the ER product should be conducted.

- A single-dose food-effect study should be conducted on the highest ER strength (see the 2002 Food-Effect Guidance).

- A steady state study should be conducted on the highest strength of the ER product compared to an approved IR reference dosed to achieve equivalent total dose as for the ER product.

<u>New ER product (ER_{new}) comparison to an approved ER product (ER_{old}) with a different dosing interval (i.e., where ER_{new} and ER_{old} have unequal dosing intervals)</u>

- The recommendations are the same as outlined in the previous section (Development of a new ER formulation given an already approved IR product) except for the choice of the reference product. In this case, the reference product could be either the approved ER_{old} or IR product.

<u>New ER product (ER_{new}) comparison to an approved ER product (ER_{old}) with the same dosing interval</u>

- A single-dose fasting BE study on the highest strength of the ER_{new} product compared to the ER_{old} product. If ER_{new} and ER_{old} are of different strength, then

either the 50-mg IR reference product administered TID or 75-mg IR reference product administered BID. In this case, the least common time interval is 24 hours.

[22] If three strengths, 10, 25, and 50 mg, are being developed for a new ER dosage form, the dosage strength equivalence study should be conducted using 5×10 mg, 2×25 mg, and 1×50 mg to achieve constancy of dose.

[23] If three strengths, 10, 25, and 50 mg, are being developed for a new ER dosage form, the dosage strength proportionality study should be conducted using 1×10 mg, 1×25 mg, and 1×50 mg to achieve constancy of dose and the dosage strength proportionality study should be conducted using 1×10 mg, 1×25 mg, and 1×50 mg.

comparison of ER_{new} versus ER_{old} should be made based on dose using the highest strengths.

- A single-dose, food-effect study should be conducted on the highest ER_{new} strength.

- When the ER_{new} strengths are not proportionally similar in composition, a single-dose fasting dosage strength equivalence assessment study or a dosage strength proportionality study[24] for the ER_{new} product should be conducted.

- In some cases, BE between the new and old ER products may not be sufficient to ensure that there is no difference in safety or efficacy if the PK profiles of the two ER products do not match (e.g., T_{max} is different). Additional data analysis or clinical studies may be needed to ensure that the two products are clinically equivalent.

2. Postapproval Changes

Information on the types of in vitro dissolution and in vivo BE studies for ER drug products approved in the presence of specific postapproval changes are provided in an FDA guidance for industry *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. We recommend that for postapproval changes, the in vitro or in vivo comparison be made between the post-change and pre-change products.

D. Batch Size

For pivotal BE studies, the test batch should be representative of the production batches. Therefore, the size of the test batch should be at least 10% of the planned production batch size, or a minimum of 100,000 units, whichever is larger.

V. ADDITIONAL INFORMATION ON IN VITRO APPROACHES

A. In Vitro Studies Conducted in Support of a Waiver of an In Vivo BA or BE Data Requirement

As discussed above, FDA's regulations contemplate that if in vivo BA or BE data are required for a product, a sponsor may seek a waiver of that requirement under certain circumstances.[25]

[24] 21 CFR 320.21(b) (giving applicants the option of submitting information that "would permit FDA to waive the submission of evidence demonstrating in vivo bioequivalence") and 320.21(f) (requiring that the information submitted in support of a waiver request "shall meet the criteria set forth in § 320.22").

[25] 21 CFR 320.21(b) (giving applicants the option of submitting information that "would permit FDA to waive the submission of evidence demonstrating in vivo bioequivalence") & 320.21(f) (requiring that the information submitted in support of a waiver request "shall meet the criteria set forth in § 320.22.")

For example, in some instances, in vivo BA or BE is self-evident based on certain characteristics of the drug product (21 CFR 320.22(b)), and therefore, any in vivo data requirement has been deemed to have been met. In other delineated circumstances, an in vivo BA or BE data requirement may be waived, and in vitro data may be accepted in lieu of in vivo data (21 CFR 320.22(d)). For example, an in vivo data requirement may be waived for different strengths of an immediate-release drug product under 21 CFR 320.22(d)(2) when (1) the drug product is in the same dosage form, but in a different strength; (2) this different strength is proportionally similar in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval; and (3) the new strength meets an appropriate in vitro test as outlined in the regulation.[26] In addition, for waiving higher strengths, linearity of the pharmacokinetics over the therapeutic dose range should be demonstrated.

This guidance defines *proportionally similar* in the following ways:

- All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).

- For high-potency drug substances (where the amount of active drug substance in the dosage form is relatively low), (1) the total weight of the dosage form remains nearly the same for all strengths (within ± 10 % of the total weight of the strength on which a BE was performed), (2) the same inactive ingredients are used for all strengths, and (3) the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients.

- Bilayer tablets are considered to be one formulation even though they consist of two separate layers with different compositions. In assessing the proportional similarity of the different strengths, all components of both layers should be proportionally similar. The fact that only one layer is proportionally similar and the other is not clearly indicates that the products (whole tablet) are not proportionally similar. This is relevant because there can be interactions between the different tablet layers, which can differ across different strengths because of the different size of the layers and the varying amounts of excipients present in each layer.

Exceptions to the above definitions may be possible if adequate justification is provided and discussed with the appropriate review division.

B. In Vitro Studies Conducted in Support of Demonstrating BA or BE

[26] See also 21 CFR 322.22(d)(3) and (4) for additional bases for waiver. Also, FDA, for good cause, may waive a requirement for the submission of evidence of in vivo bioavailability or bioequivalence if waiver is compatible with the protection of the public health. For full NDAs, FDA may defer a requirement for the submission of evidence of in vivo bioavailability if deferral is compatible with the protection of the public health (21 CFR 320.22(e)).

FDA may determine that in vitro data are the most accurate, sensitive, and reproducible method to demonstrate BA or BE in other contexts (21 CFR 320.24(b)(5) and (6)).[27] Below we provide additional guidance on the conduct of such studies.

1. Immediate-Release Formulations (Capsules, Tablets, and Suspensions)

In vitro data can be used to compare formulations of drug products under certain circumstances. If an applicant seeks to demonstrate the BA or BE of immediate-release formulations for capsules, tablets, and suspensions using in vitro data, FDA recommends that sponsors generate dissolution profiles for all strengths using an appropriate dissolution method. If the dissolution results indicate that the dissolution characteristics of the product are not dependent on the pH and product strength, dissolution profiles in one medium are usually sufficient to support demonstrating BE. Otherwise, dissolution data in at least three media (e.g., pH 1.2, 4.5, and 6.8) are recommended. The f_2 test should be used to compare profiles from the different strengths of the product (see FDA guidance for industry, *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*). An f_2 value ≥ 50 indicates a sufficiently similar dissolution profile to support a biowaiver. For an f_2 value < 50, discussion with the appropriate review division is recommended to determine whether an in vivo study is needed. The f_2 approach is not suitable for rapidly dissolving drug products (e.g., $\geq$ 85% dissolved in 15 minutes or less).

- *Over-encapsulation of clinical trial formulations*

During the course of drug development, sponsors sometimes have to blind the formulations that they use in the clinical trials. In certain situations, the only difference between the to-be-marketed and clinical trial formulations is that the dosage form is put into a capsule. This over-encapsulation is done mainly for blinding purposes. It may be possible to support bioequivalence of the to-be-marketed and clinical trial formulations using in vitro data only, provided that no other excipients are added to the capsule and the dissolution profiles are comparable in three media: pH 1.2, pH 4.5 and pH 6.8.

- *Scale-up and postapproval changes*

Certain formulation changes in components and composition, scale-up, manufacturing site, manufacturing process, or equipment can be made postapproval. Depending on the possible impact of the manufacturing change on the release of the active ingredient from the formulation and its BA, certain manufacturing changes for IR products can be approved based solely on similarity of the dissolution profiles between the postchange and prechange formulations. Information on recommendations for using in vitro dissolution and in vivo BE studies for immediate-release drug products in such circumstances is provided in FDA's guidance for industry on *SUPAC IR: Immediate-Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence*

[27] In such instances, no waiver under 21 CFR 320.21 and 320.22 is necessary.

Documentation. The same principles described in the guidance can be applied to pre-approval changes in which the to-be-marketed formulation differs from the clinical trial formulation.

2. Modified-Release Formulations

The use of in vitro data may be acceptable for modified-release drug products for which specific postapproval changes are sought is delineated in the FDA guidance for industry *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation.* The same principles described in the guidance may also apply to preapproval changes. Additional considerations for use of in vitro data are described below.

- *Beaded capsules: lower/higher strength*

For ER beaded capsules where the strength differs only in the number of beads containing the active moiety, a single-dose, fasting BA or BE study, as appropriate, should be carried out on the highest strength. In vivo BA or BE of one or more lower strengths can be demonstrated based on dissolution profile comparisons, with an in vivo BA or BE study only on the highest strength (unless safety reasons preclude the administration of the highest strength to healthy volunteers). The dissolution profiles for each strength should be generated using the recommended dissolution method. If the dissolution method has not been finalized, dissolution profiles should be generated in at least three media (e.g., pH 1.2, 4.5, and 6.8). In vivo BE studies for higher strengths may not be necessary based on (1) clinical safety and/or efficacy data on the proposed dose and the need for the higher strength, (2) linearity of pharmacokinetics over the therapeutic dose range, and (3) the same dissolution procedures being used for all strengths with similar dissolution results. The f_2 test can be used to demonstrate similar profiles among the different strengths of the product.

- *MR dosage forms: lower strength*

For MR dosage forms, when the drug product is in the same dosage form but in a different strength and when (1) the drug exhibits linear pharmacokinetics, (2) the various strengths are proportionally similar in their active and inactive ingredients[28] and (3) the drug-release mechanism is the same, an in vivo BA or BE determination of one or more lower strengths can be demonstrated based on dissolution profile comparisons, with an in vivo BA or BE study only on the highest strength. The dissolution profiles for each strength should be generated using the recommended dissolution method. If the dissolution method has not been finalized, dissolution profiles should be generated in at

[28] If the formulations of all the strengths are not compositionally proportional, in vitro data can be submitted for the middle strength(s) if the following data are acceptable: (1) BA or BE data, as appropriate, for both the highest and the lowest strengths, and (2) in vitro multimedia dissolution comparison profiles using f2 evaluation.

least three media (e.g., pH 1.2, pH 4.5, and pH 6.8). The dissolution profile should be generated on the test and reference products of all strengths using the same dissolution test conditions.

VI. SPECIAL TOPICS

A. Alcoholic Beverage Effects on MR Drug Products

The consumption of alcoholic beverages may affect the release of a drug substance from an MR formulation. The formulation may lose its MR characteristics, leading to more rapid drug release and altered systemic exposure. This more rapid drug release may have deleterious effects on the drug's safety and/or efficacy.

In vitro assessments of the drug release from the drug product using media with various alcohol concentrations should be conducted. Based on the results of the in vitro assessments, an in vivo BA study of the drug product when administered with alcohol may be needed.

B. Enantiomers versus Racemates

During development of a racemic drug product, the racemate should be measured in BA studies. It may also be important to measure the individual enantiomers of the racemate to characterize the pharmacokinetics of the enantiomers. For the development of a specific enantiomer, chiral inversion should be assessed.

Measurement of the racemate using an achiral assay is recommended for BE studies. Measurement of individual enantiomers in BE studies is recommended only when all of the following conditions are met: (1) the enantiomers exhibit different PD characteristics, (2) the enantiomers exhibit different PK characteristics, (3) primary efficacy and safety activity resides with the minor enantiomer, and (4) nonlinear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for at least one of the enantiomers. In such cases, we recommend that BE criteria be applied to the enantiomers separately.

C. Drug Products With Complex Mixtures as the Active Ingredients

Certain drug products may contain complex drug substances (i.e., active moieties or active ingredients that are mixtures of multiple synthetic and/or natural source components). Some or all of the components of these complex drug substances may not be fully characterized with regard to chemical structure and/or biological activity. Quantification of all active or potentially active components in BA and BE studies may not be possible. In such cases, we recommend that BA and BE studies be based on a select number of components. Criteria for component selection typically include the amount of the moiety in the dosage form, plasma or blood levels of the moiety, and biological activity of the moiety. When PK approaches are infeasible to assess rate and extent of absorption of a drug substance from a drug product, PD, clinical, or in vitro approaches may be appropriate.

D. Long-Half-Life Drugs

In a BA or PK study involving an IR oral product with a long half-life (≥ 24 hours), adequate characterization of the half-life should include blood sampling over a long period of time. For BA or BE determination of a drug product containing a drug with a long half-life, a nonreplicate, single-dose, crossover study can be conducted, provided an adequate washout period is used. If the crossover study is problematic, a study with a parallel design can be used. For either a crossover or parallel study, we recommend that the sample collection time be adequate to ensure completion of gastrointestinal transit (approximately 2 to 3 days) of the drug product and absorption of the drug substance. C_{max} and a suitably truncated AUC can be used to characterize peak and total drug exposure, respectively. For drugs that demonstrate low intrasubject variability in distribution and clearance, a truncated AUC (e.g., $AUC_{0\text{-}72\ hr}$) can be used in place of $AUC_{0\text{-}t}$ or $AUC_{0\text{-}\infty}$. For drugs that demonstrate high intrasubject variability in distribution and clearance, AUC truncation should not be used. In such cases, we recommend that sponsors and/or applicants consult the appropriate review division.

E. Orally Administered Drugs Intended for Local Action

Documentation of BA and BE when the drug substance produces its effects by local action in the gastrointestinal tract can be achieved either by using pharmacokinetics, an acceptable PD end point, clinical efficacy and safety studies, and/or suitably designed and validated in vitro studies, as appropriate. For such cases, we recommend that sponsors and/or applicants consult the appropriate review division. Additional safety studies may also be recommended to characterize the local safety of the product. The in vitro studies should reflect important clinical effects or should be more sensitive to changes in product performance compared to a clinical study. To ensure comparable safety, additional studies with and without food may help to understand the degree of systemic exposure that occurs following administration of a drug product intended for local action in the gastrointestinal tract.

F. Combination/Coadministered Drug Products

Two or more active ingredients can be formulated as a single drug product, which is referred to as a combination drug product. Generally, the purpose of an in vivo BA study involving a combination drug product is to compare the rate and extent of absorption of each active drug ingredient or therapeutic moiety in the combination drug product to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently in separate single-ingredient preparations (21 CFR 320.25(g).

For the purpose of defining BA or determining BE when required, this guidance recommends that the following studies be conducted for a combination drug product:

- A two-treatment, single-dose, fasting study of the combination drug product versus single-ingredient drug products administered concurrently as a single treatment or an approved combination product containing the same active ingredients. This study should

use the highest strength of the combination product with matching doses of individual drug products.

- Certain alternative study designs may also be acceptable depending on the specific situation. For instance, in the case of a combination product consisting of two components, a three-treatment study design comparing the combination drug product versus single-ingredient drug products administered separately may be appropriate.

- A single-dose, food-effect study on the combination drug product.

BE studies for the combination product should include the measurement of systemic concentrations of each active ingredient. The confidence interval approach should be applied to each measured entity of the combination drug product and its reference product.

In specific cases, drug products are given in combination (not co-formulated) with the objective of increasing the exposure of one of the drugs (subject drug). The second drug is not intended to have a therapeutic effect and is given only to increase the systemic exposure of the subject drug. When both the subject and second drug are new molecular entities, the BA of each should be assessed separately. If a BE study is needed for the subject drug for any reason, the subject drug should be administered with the second drug for both test and reference products. The corresponding PK results, including confidence intervals for BE criteria, should be applied to the subject drug. It is not necessary to measure the concentrations of the second drug. BE studies that are needed for the second drug should be conducted only with the second drug; the subject drug is not dosed with the second drug. When the combination includes a new molecular entity and an approved product, only the BA of the new molecular entity should be assessed. It is assumed that the BA of the approved product has been previously evaluated.

G. Endogenous Substances

Drug products can be developed that contain compounds that are endogenous to humans (e.g., testosterone). When the endogenous compounds are identical to the drug that is being administered, determining the amount of drug released from the dosage form and absorbed by each subject is difficult. In most cases, it is important to measure and approximate the baseline endogenous levels of the compound in blood (plasma) and subtract these levels from the total concentrations measured from each subject after the drug product is administered. In this way, an estimate of actual drug availability from the drug product can be achieved, and therefore BA and BE can be assessed. Endogenous substances may have homeostatic processes that affect their production and therefore impact their systemic concentrations. To reduce the complication of these homeostatic processes and to potentially avoid the need for baseline correction, an alternative approach might be to enroll patients in BA and BE studies with low or no production of the endogenous substances instead of healthy volunteers.

Baseline concentrations of the endogenous substance produced by the body are measured in the time period prior to study drug administration. Depending on the proposed indication, subtraction of the time-averaged baseline or time-matched baseline from the post-dose

concentration for each subject may be recommended. When the endogenous levels are influenced by diet, strict control of the dietary intake of the compound prior to and during the study may also be appropriate. To achieve a stable baseline, subjects should be housed at the clinic for a sufficient time prior to the study and served standardized meals with similar content of the compound to that of the meals served on the PK sampling day.

In either case, baseline concentrations should be determined for each dosing period, and baseline corrections should be period-specific. If a negative plasma concentration value results after baseline correction, this should be set to 0 prior to calculating the baseline-corrected AUC. Pharmacokinetics and statistical analysis should be performed on both uncorrected and corrected data as appropriate. Because of the complexities associated with endogenous compounds, we recommend that sponsors and/or applicants contact the appropriate review division for additional guidance.

H. Drug Products With High Intrasubject Variability

In addition to the traditional approach and the use of average BE using replicate designs, the use of a reference-scaled BE approach using a replicate design can be considered. This approach should be reserved for drugs that demonstrate a high intrasubject variability (≥30%). The reference-scaled average BE approach adjusts the BE limits of highly variable drugs by scaling to the within-subject variability of the reference product in the study and imposes a limit of 0.8 to 1.25 on the geometric mean ratio.[29] The appropriate review division should be consulted when planning the use of the reference-scaled BE approach.

[29] For general principles of the reference-scaled approach, refer to Davit B, Conner D. Reference-Scaled Average Bioequivalence Approach. In: Kanfer I, Shargel L, Eds. *Generic Drug Product Development – International Regulatory Requirements For Bioequivalence.* Informa Healthcare, 2010:271-272.

APPENDIX A: GENERAL STUDY DESIGN AND DATA HANDLING

The following general approaches are recommended, recognizing that the elements can be adjusted for certain drug substances and drug products.

Study conduct

- The BA or BE study should be conducted under fasting conditions (after an overnight fast of at least 10 hours). If the BA or BE study needs to be conducted with food, a separate FDA guidance *Food-Effect Bioavailability and Fed Bioequivalence Studies* is available to assist sponsors.

- The test and reference products should be administered with about 8 ounces (240 milliliters) of water to an appropriate number of subjects.

- Generally, the highest marketed strength should be administered as a single unit. If warranted, to achieve sufficient bioanalytical sensitivity multiple units of the highest strength can be administered, provided the total single dose remains within the labeled dose range and the total dose is safe for administration to the study subjects.

- An adequate washout period (e.g., ≥5 half-lives of the moieties to be measured) should separate each treatment.

- The lot numbers of both test and reference listed products and the expiration date for the reference product should be stated. We recommend that the assayed drug content of the test product batch not differ from the reference product by more than +/- 5 percent. The sponsor should include a statement of the composition of the test product and, if possible, a side-by-side comparison of the compositions of test and reference listed products. In accordance with 21 CFR 320.38, and 21 CFR 320.63, samples of the test and reference listed product must be retained for at least 5 years. For additional information, please refer to the FDA guidance for industry on *Handling and Retention of Bioavailability and Bioequivalence Testing Samples.*

- Before and during each study phase, we recommend that subjects (1) be allowed water as desired except for 1 hour before and after drug administration, (2) be provided standard meals no less than 4 hours after drug administration, and (3) abstain from alcohol for 24 hours before each study period and until after the last sample from each period is collected.

Sample collection and sampling times

- We recommend that under normal circumstances, blood, rather than urine or tissue, be used. In most cases, drug or metabolites are measured in serum or plasma. However, in certain cases, such as when an assay of sufficient sensitivity cannot be developed for plasma, whole blood may be more appropriate for analysis. We recommend that blood samples be drawn at

appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs we recommend that 12 to 18 samples, including a pre-dose sample, be collected per subject per dose. ***This sampling should continue for at least three or more terminal elimination half-lives of the drug*** to capture 90 percent of the relevant AUC. For multiple-dose studies, sampling should occur across the dose interval and include the beginning and the end of the interval. The exact timing for sample collection depends on the nature of the drug and the rate of input from the administered dosage form. The sample collection should be spaced in such a way that the maximum concentration (C_{max}) of the drug in the blood and terminal elimination rate constant (λ_Z) can be estimated accurately.

Three or more samples should be obtained during the terminal log-linear phase to obtain an accurate estimate of λ_z from linear regression. We recommend recording the actual clock time when samples are drawn, as well as the elapsed time related to drug administration.

Subjects with pre-dose plasma concentrations

- If the pre-dose concentration is ≤ 5 percent of C_{max} value in that subject, the subject's data without any adjustments can be included in all PK measurements and calculations. We recommend that if the pre-dose value is > 5 percent of C_{max}, the subject should be dropped from all PK evaluations. The subject data should be reported and the subject should be included in safety evaluations.

Data deletion because of vomiting

- We recommend that data from subjects who experience emesis during the course of a study for immediate-release products be deleted from statistical analysis if vomiting occurs at or before 2 times median T_{max}. For modified-release products, subjects who experience emesis at any time during the labeled dosing interval should not be included in PK analysis.

Data submission and analysis

The following PK information is recommended for submission:

- Plasma concentrations and time points.
- Subject, period, sequence, treatment.
- Intersubject, intrasubject, and/or total variability, if available.
- For single-dose studies: $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max}, λ_z, and $t_{1/2}$.
- For steady-state studies: $AUC_{0\text{-}tau}$, C_{maxss}, T_{max}, C_{minss} (lowest concentration in a dosing interval), C_{trough} (concentration at the end of the dosing interval), C_{avss} (average concentration during a dosing interval), degree of fluctuation $[(C_{max}\text{-}C_{min})/C_{avss}]$, swing $[(C_{maxss}\text{-}C_{minss})/C_{minss}]$. C_{trough} should be measured for several dosing intervals to assess whether steady-state was achieved.

- In addition to the above information, clearance and volume of distribution should be reported for BA studies.

In addition, we recommend that the following statistical information be provided for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}:

- Geometric means
- Arithmetic means
- Geometric mean ratios
- 90 percent Confidence intervals (CI)

We also recommend that logarithmic transformation be provided for measures used for BE demonstration. An FDA guidance for industry, *Statistical Approaches to Establishing Bioequivalence*, is available.

Rounding off of confidence interval values

We recommend that applicants ***not round off*** CI values; therefore, to pass a CI limit of 80 to 125 percent, the value should be at least 80.00 percent and not more than 125.00 percent.